AWARD NUMBER:
W81XWH-16-1-0556

TITLE:
Stress Hormone Enhancement of OP-Induced Neuroinflammation as an Animal Model of GWI: The Role of Toll-like Receptors and Plasticity

PRINCIPAL INVESTIGATOR:
Stephen M. Lasley, Ph.D.

CONTRACTING ORGANIZATION:
Office of Research Services
Univ. of Illinois at Chicago
304 AOB, M/C 672
1737 West Polk Street
Chicago, IL 61612-7227

REPORT DATE:
September 2017

TYPE OF REPORT:
Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
~~30-09-2017~~xxxx Sept 2017	Annual	31 Aug 2016 – 30 Aug 2017

4. TITLE AND SUBTITLE

Stress Hormone Enhancement of OP-Induced Neuroinflammation as an Animal Model of GWI: The Role of Toll-like Receptors and Plasticity

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-16-1-0556

5c. PROGRAM ELEMENT NUMBER

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

6. AUTHOR(S)

Stephen M. Lasley, Ph.D.

E-Mail: SML@uic.edu

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Univ. of Illinois at Chicago
Business Affairs
809 S. Marshfield, Rm 608
Chicago, IL 60612-7205

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Gulf War Illness (GWI) is a multi-symptom disorder with features similar to "sickness behavior" (e.g., fatigue, joint pain, cognitive impairments, gastrointestinal problems). The exposures and conditions in theater that caused GWI remain unknown but several chemicals and environmental conditions have been implicated. In addition, we have combined GW agent exposures with corticosterone (CORT) in a mouse model to simulate physiological stresses in the war theater. Project leaders decided that the behavioral studies should be conducted before the neurophysiological and biochemical work to establish a CORT+CPO regimen that produced a defined functional effect. The Morris water maze was the initial behavioral test of spatial learning employed. Separate groups of mice were tested 1 and 12 weeks after CORT + CPO exposure, but there was no evidence that this treatment produced an impairment of learning in the water maze. It was therefore apparent that the priming of the immune response from one week of CORT administration plus the neuroinflammation produced by CPO one day later was not sufficient to affect learning and memory assessed in the water maze. In year 2 we will include additional behavioral tests to better validate the presence of any treatment-related behavioral effects. In addition, we will utilize a longer CORT regimen with the same dose of CPO, but implement a standardized immune stimulus just prior to behavioral testing. These latter steps are designed to enhance the resulting neuroinflammation.

15. SUBJECT TERMS

Gulf War Illness, chronic neuroinflammation, chlorpyrifos oxon, physiological stress, Morris water maze, lipopolysaccharide

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	10	19b. TELEPHONE NUMBER *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Table of Contents

INTRODUCTION

Gulf War Illness (GWI) is a multi-symptom disorder with features similar to "sickness behavior" (e.g., fatigue, joint pain, cognitive impairments, gastrointestinal problems). While these symptoms usually abate over time as physiological homeostasis returns, individuals with GWI experience recurring bouts of severe symptoms. The exposures and conditions in theater that caused GWI remain unknown but several chemicals and environmental conditions have been implicated. In addition, we have combined GW agent exposures with corticosterone (CORT) in a mouse model to simulate physiological stresses in the war theater. We have previously determined that proinflammatory effects of DFP were markedly enhanced by pretreatment with the anti-inflammatory stress hormone CORT. The neuroinflammatory effects observed after CORT + DFP exposure consisted of increased elaboration of proinflammatory cytokines and chemokines, providing the underlying molecular basis for "sickness behavior". This project will extend these key findings with DFP to the additional GWI-relevant OPs, chlorpyrifos (CPF) and dichlorvos (DDVP). Also, a greater understanding of the basis of the CORT "priming" effect is needed to identify targets for therapeutic intervention. Toll-like receptor 2 (TLR2) pathways have been implicated in the signaling underlying neuroinflammatory "priming", and accordingly the project will investigate the role of TLR2 in the development of CORT+OP-induced neuroinflammation. Finally, experiments will define the extended duration of the synaptic and behavioral effects resulting from CORT+OP-induced neuroinflammation and means to diminish this condition with pharmacological trophic factors that affect neurogenesis and plasticity. The impact of confirming a role for these mechanisms in our GWI model will be invaluable to the understanding of the molecular basis of GWI for targeted therapeutic interventions.

KEYWORDS

Gulf War Illness	chronic neuroinflammation	chlorpyrifos oxon
physiological stress	Morris water maze	hippocampus
neurogenesis	synaptic plasticity	neurotrophins
corticosterone priming	inflammatory mediators	gene expression
neurophysiology		

ACCOMPLISHMENTS

After some discussion at the beginning of the project Drs. O'Callaghan, Miller, and Lasley decided that the behavioral studies (Statement of Work (SOW) Major Task 7) should be conducted before the neurophysiological (SOW Major Task 6) and biochemical (SOW Major Tasks 5-6) work to establish a CORT+OP regimen that produced a defined functional effect. This approach was important since behavioral experiments preceding this project being funded had not been successful in this pursuit. Once established, this treatment regimen would then be the basis of the remaining studies conducted on the project at the University of Illinois at Chicago.

The Morris water maze was the initial behavioral test of spatial learning employed instead of the Barnes maze (also a spatial learning paradigm) specified in Major Task 7. There is a wealth of information in the biomedical literature concerning execution and scoring of this test with

rodents – much more than for the Barnes maze – and we had previous experience with this paradigm. In this test a mouse must learn the location of an escape platform by swimming in a pool of water at room temperature and using cues in the test environment. The platform sits just below the water surface, and is not visible because the water is made opaque with powdered milk. Acquisition training was conducted over 6 days with four trials per mouse per day with trials separated by 30-40 minutes. After every 8 trials (i.e., every other day) mice are given a probe trial in which the hidden platform is removed from the pool. A video camera records each trial and animal performance is scored by commercial software that measures latency to find the platform, time spent in the platform quadrant of the pool, and the mean distance to the platform.

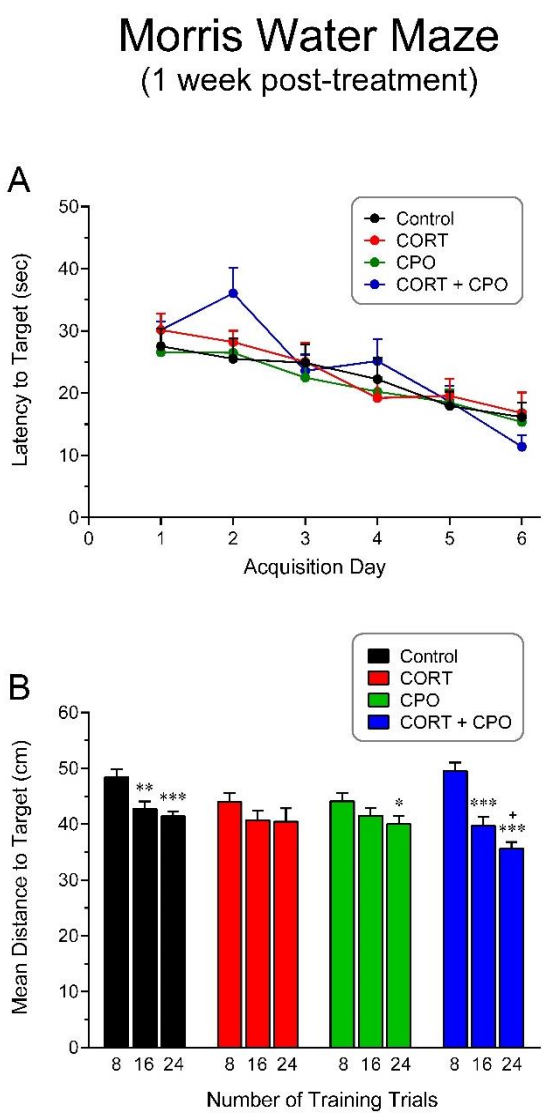

Figure 1. Water maze test results conducted 1 week after CORT + CPO treatment. Latencies (A) and Mean Distance to Target (B) decrease as mice learn location of escape platform (N = 12-14/group). No between group differences were found. $^{**}p < 0.01$; $^{***}p < 0.001$ vs. corresponding mean for 8 trials. $^{+}p < 0.05$ vs. mean for 16 trials.

Mice were administered CORT in their drinking water at 0.4 mg/ml for 7 days and on day 8 chlorpyrifos oxon (CPO) was administered at 2 mg/kg, i.p. The study included four experimental groups: a vehicle Control group, groups receiving only CORT or only CPO, and a group treated with both CORT plus CPO (N = 12-14 mice/group). Testing in the water maze began one week later. The results are shown in Figure 1A for the latency measure, which clearly decreased across acquisition trials as the animals learned the hidden platform location (the Trials $F = 18.57$, $p < 0.0001$). We have found the mean distance to target to be the most reliable indicator of learning, and Figure 1B demonstrates that learning took place in all groups except for the CORT only group (Treatment $F = 47.10$, $p < 0.0001$; Treatment × Trials interaction $F = 4.96$, $p < 0.0002$). These data indicate that the CORT+CPO regimen did not impair learning in the water maze.

In consideration of the possibility that a cognitive impairment may take longer than one week to develop after CORT+CPO exposure, a small cohort of mice (N = 6/group) were administered the same exposure regimen described above, but 86 days were allowed to transpire between CPO administration and initiation of acquisition training for the water maze. The results of testing are shown in Figure 2. Again, there is a decrease in the latency to find the platform across acquisition days in all groups in Figure 2A (Trials $F = 24.94$, $p < 0.0001$), and performance on the probe tests in

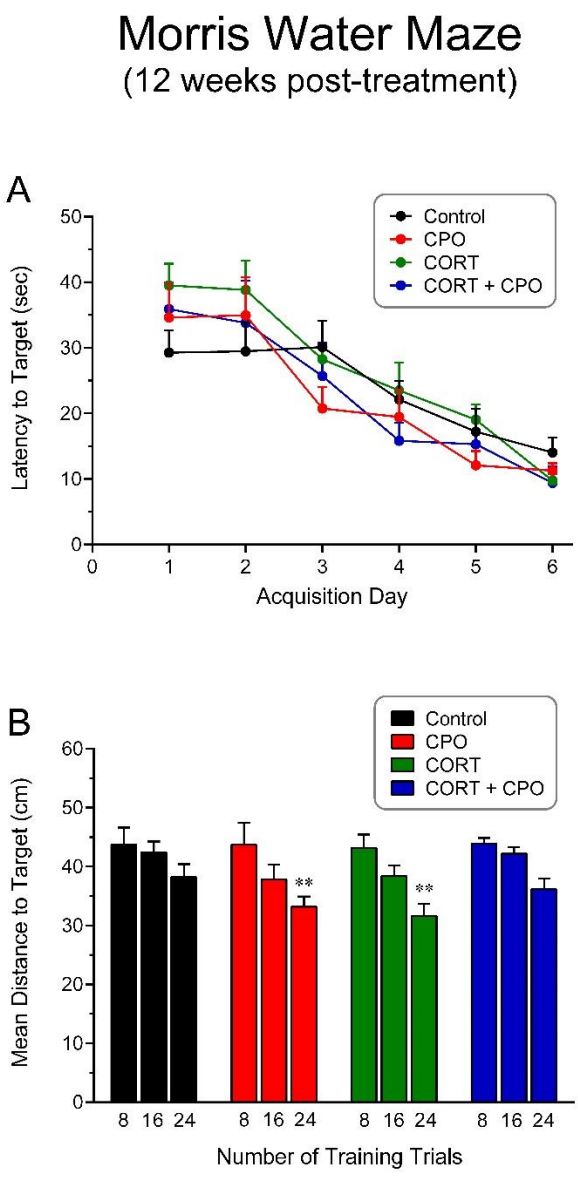

Figure 2. Water maze test results conducted 12 weeks after CORT + CPO treatment. Latencies (A) and Mean Distance to Target (B) decrease as mice learn location of the escape platform (N = 6-8/group). No between group differences were found. $^{**}p < 0.01$ vs. corresponding mean for 8 trials.

Figure 2B demonstrate that spatial learning would likely have occurred in all groups had a full cohort (N = 6-8/group) been tested (Trials $F = 16.11$, $p < 0.0001$). Again, there was no evidence that the CORT+CPO treatment produced an impairment of learning in the water maze.

It was therefore apparent that the priming of the immune response from one week of CORT administration plus the neuroinflammation produced by CPO one day later was not sufficient to affect learning and memory assessed in the water maze one or twelve weeks post-administration.

We have consequently planned some changes to our approach to pursue in year 2 of the project and the revisions will be submitted shortly for ACURO approval. In one respect, we will increase the number of behavioral tests performed to account for the possibility that the plasticity changes produced by the one week CORT plus CPO regimen could not be discriminated by a spatial learning paradigm, or that an effect on learning and memory had dissipated by 1-2 weeks post-CPO administration when water maze testing was conducted. Multiple tests will also better validate the presence of any treatment-related behavioral effects. We have therefore established two other test paradigms in the lab – novel object recognition (NOR) and novel object location (NOL) – to test the mice in addition to the water maze. In these tests the animals are given habituation sessions in the test chamber with two similarly sized objects present. On test days one of the two familiar objects is replaced with a novel one (NOR test) or one of the familiar objects is moved to a new location in the chamber (NOL test). A video camera records the animals' movements in the test chamber during the tests, and animals are scored on the time they spend exploring the novel object or the familiar object in a new location. We have used pilot animals from another local animal care protocol to set up these tests, and the data shown in Figure 3 demonstrates that these setup efforts were successful (on test day for NOR: *t*-test = 5.82, $p < 0.0001$; for NOL: *t*-test = 2.52, $p =$

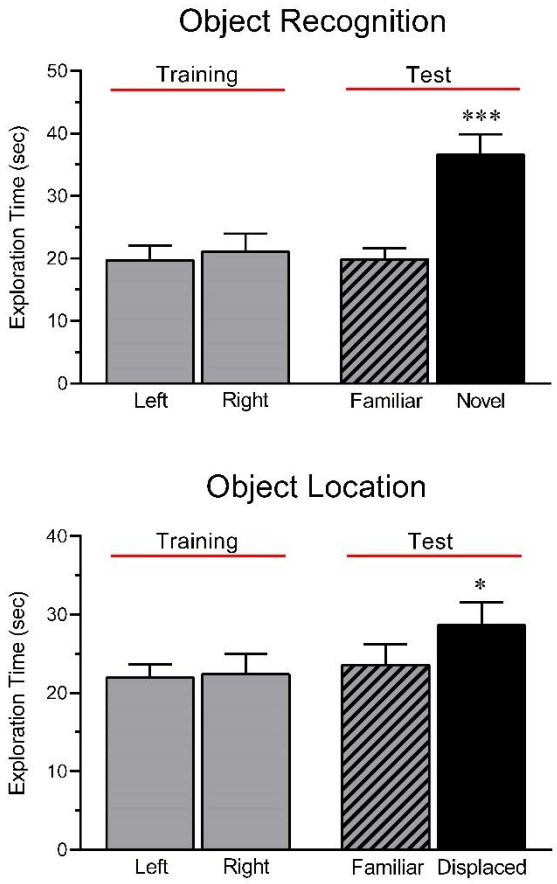

Figure 3. Exploration times of untreated pilot mice in the NOR and NOL test paradigms (N = 6). After training sessions with two objects in the same test chamber, on test day mice discriminate a novel object or a familiar object displaced to a novel location. *$p < 0.05$; ***$p < 0.001$ vs. exploration of novel object/location on test day.

0.029; N = 12). Water maze learning is widely thought to be hippocampally mediated, but requires 10-12 days to complete and may miss transient effects of neuroinflammation. The novel object tests require only 2-3 days to execute and can therefore cover the shorter time intervals after the treatment regimen is complete. In addition, they complement the water maze test, as the NOL also is thought to rely on hippocampal function while performance in the NOR is considered to be more globally represented in the brain.

It was also reasoned that to achieve sufficient neuroinflammation to induce behavioral changes it may be necessary to: 1) enhance CORT priming in the animals by administration for longer than one week; 2) administer a standard immune stimulus at some time point after CPO to induce a renewed neuroinflammation. Thus, in consultation with Dr. Jim O'Callaghan at the Centers for Disease Control and Prevention we developed a 5-week intermittent CORT administration regimen in which CORT was administered in the drinking water during weeks 1, 3, and 5. CPO would be administered after week 1 of CORT as in the shorter regimen, and then a low dose of lipopolysaccharide (LPS) would be injected at the end of week 5 with behavioral testing beginning within the next few days. These are the studies that will be undertaken at the beginning of year 2 of the project, and will be followed by the neurogenesis experimentation (SOW Major Task 5).

During year 1 of the project Raghava Sriramaneni, B.S., worked for about three months contributing to the execution of the water maze testing before moving to Wisconsin. This was his first exposure to behavioral testing and allowed him to extend his research experience in a new direction. Catherine McCarthy, a junior pre-med major from Eureka College, worked on the project during the summer of 2017. This period was among her first experiences in laboratory research, as she also contributed to the behavioral testing and scoring.

IMPACT

There were no experimental findings in year 1 of the project that had a significant impact on neurotoxicology, largely because of the negative results described above. The primary impact was directed to the CORT + CPO mouse model and the inability to define an effect on spatial

learning and memory after one week of CORT exposure. This resulted in the design of a revised treatment regimen that entailed longer CORT exposure and presentation of an immune stimulus along with additional behavioral test paradigms.

CHANGES/PROBLEMS

There are no significant changes to the project that will occur in year 2. Rather the revisions described in the final paragraphs of Accomplishments are manipulations of treatment parameters and also involve inclusion of additional test paradigms. The negative results in year 1 and the longer duration CORT + CPO protocol will make achieving progress more of a challenge, but we are hopeful of still completing all work proposed in the SOW. Approval for the experimental design changes affecting the animals have been approved by the local IACUC and will be sought from ACURO shortly.

At the dose utilized (0.25 mg/kg, i.p.) LPS is not considered a hazardous chemical and will not pose a danger of lethality to the animals.

A more detailed description of the revisions to the Research Plan and the basis for the modifications is provided in the latter paragraphs of Accomplishments.

PRODUCTS

Nothing to report

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

The following individuals participated in the project in year 1.

Name: Stephen M. Lasley, Ph.D.
Project Role: Principal Investigator
Researcher Identifier:
Nearest Person Months Worked: 12
Contribution to Project: Dr. Lasley directed all studies in the project in consultation with Dr. Fornal (see below) and Dr. O'Callaghan at CDC.
Funding Support: Salary support from the State of Illinois

There has been no change in Dr. Lasley's active Other Support.

Name: Casimir A. Fornal, Ph.D.
Project Role: Research Scientist
Research Identifier:
Nearest Person Months Worked: 12
Contribution to Project: Conducts all studies under Dr. Lasley's direction; administers all components of treatment regimen. Performs data reduction for presentation and review.
Funding Support:

Name: Raghava Sriramaneni, B.S.
Project Role: Research Technician
Research Identifier:
Nearest Person Months Worked: 3 (part-time)
Contribution to Project: Assisted with behavioral (water maze) experiments
Funding Support:

Name: Catherine McCarthy
Project Role: Research Technician
Research Identifier:
Nearest Person Months Worked: 3 (part-time)
Contribution to Project: Assisted with behavioral experiments
Funding Support:

The following organization was involved in this project as a partner organization with independent funding as part of this Research Expansion Award.

Organization Name: Centers for Disease Control and Prevention
Location of Organization: Morgantown, West Virginia
Partner's Contribution to the Project:
Collaboration – collaboration through Dr. James P. O'Callaghan and his research group

SPECIAL REPORTING REQUIREMENTS
A collaborative award has been made to Dr. O'Callaghan for this Research Expansion Award, and an independent annual report will be forthcoming from his research group.

APPENDICES
None